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# CORRESPONDENCE

## Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia after first relapse

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TO THE EDITOR

Recently, Coustan-Smith *et al*[1] presented data on the prognostic impact of minimal residual disease (MRD) in children with relapsed acute lymphoblastic leukemia (ALL). In front-line trials, this method is already being used for patient stratification, and also in the current trial for relapsed ALL, ALL-REZ BFM 2002, the level of MRD at day 36 is decisive for the indication of stem cell transplantation (SCT) in intermediate risk patients.[2] In this particular group, a reliable prediction of subsequent relapse is crucial to allow the allocation of patients to treatment regimens of adequate efficacy and justifiable toxicity. Coustan-Smith and co-workers assessed MRD prospectively after remission reinduction using flow cytometry in a cohort of patients with first relapse of ALL. The authors confirm the prognostic value of MRD after induction therapy in children with ALL relapse, in accordance with our data on a retrospective study using molecular genetic methods.[2]

The group of Coustan-Smith and Campana[3] pioneered the establishment of flow cytometry as specific and sensitive MRD quantification method used in several large prospective MRD studies in newly diagnosed ALL. Moreover, they published recently a large-scale comparative analysis on flow cytometry and PCR for the detection of MRD, showing good concordance and recommending a tandem application.[4] In Europe, molecular genetics is most widely used in a number of countries for MRD-quantification in ALL, and a co-operative group has been established to set standards for quality control in the different laboratories. This is essential if a method is being applied in several institutions or reference laboratories in order to get reliable and comparable results. The ALL-BFM Study Group is conducting a prospective trial, evaluating the feasibility and prognostic value of flow cytometry besides PCR-based MRD-quantification in a multicentre setting. Among the European groups, the sensitivity of flow cytometry using aberrant or 'leukemia-associated' immunophenotypes is controversially discussed, in particular concerning the separation of malignant from normal B-cell precursor (BCP) cells in regenerating bone marrow (BM).[5,6] However, in the BFM study, two fixed marker combinations for MRD detection in BCP-ALL are used, whereas Coustan-Smith and co-workers employ individual marker combinations depending on the specific immunophenotype of the patients including innovative leukemia-specific markers.[3] In the multicentre setting, the cost-effectiveness is an important argument in favour of flow cytometry; furthermore, this technique is less time consuming.[5] Nevertheless, for the time being, PCR-based quantification of TCR and Ig gene rearrangements is the only technique, which is already being used to stratify patients in European trials for treatment of childhood ALL at primary manifestation or at relapse.[7]

Coustan-Smith *et al* found in a multivariate analysis both time point of relapse as well as MRD to be significant predictors of outcome, and the authors describe that the prognosis was poor in children with early relapses irrespective of the MRD level. In the BFM relapse trials we have always found that chemotherapy was not a curative treatment in children with early systemic relapse, in particular concerning T-lineage and/or isolated BM relapse, and that only SCT could offer the chance of stable and long-lasting remissions in these patients.[8] This was the reason to focus on intermediate risk patients, when investigating MRD as a prognostic parameter, that could define subgroups of patients eligible for SCT. This strategy is now confirmed by the prospective data from Coustan-Smith *et al*. However, the fact that the time of relapse, the lineage and *TEL/AML1* rearrangements were equally distributed between the MRD low and high level groups in the series published by Coustan-Smith indicates that MRD as an early response parameter may be less informative in high-risk than in intermediate risk patients. In high-risk patients, MRD proved to be an important prognostic parameter at a later time-point, prior to SCT: persisting high levels of MRD have been associated with high rates of subsequent relapse after transplantation.[9]

It is interesting to note that MRD seems to be a stable prognostic parameter irrespective of the treatment design: whereas the St Jude treatment protocols for relapsed ALL start with a continuous therapy, the ALL-REZ BFM protocols 90/96 start with intensive multiagent alternating 1-week chemotherapy courses (F1, F2, R1, R2) with 1–2 week intervals. Although intensity and design of induction protocols must have an important influence on treatment response and the dynamics of MRD, the biology of the disease proves to be the dominant factor in terms of prognosis. Nevertheless, MRD results should only be interpreted in context with the respective treatment regimen.

The a.m. differences between the two published studies on MRD in childhood relapsed ALL may explain the different MRD-levels of 0.01% (ie $10^{-4}$) in the analysis of Coustan-Smith and 0.1% ($10^{-3}$) in the BFM study, which have been detected as optimal prognostic discriminators.

In patients with isolated extramedullary relapse, the prognostic value of MRD involvement in BM at relapse and MRD reduction during therapy has not been assessed until now. As done in a subanalysis by Coustan-Smith, these patients should be regarded separately from those with BM involvement, because tumor burden at relapse is one to four logs lower than in medullary relapses, and the clinical relevance of MRD results is unclear.[10]

A very important issue is the risk of clonal evolution with respect to PCR-based methods and intra-/interlineage shifts with respect to flow cytometric methods. In particular, in isolated extramedullary relapse, material for MRD detection may not

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always be available and then marker combinations from primary disease have to be employed. In this context, it would be helpful to communicate data on stability of marker combinations at initial diagnosis and at relapse. Furthermore, the number of marker combinations used for MRD quantification should be mentioned.[3]

Irrespective of methodological differences in several study groups, it seems obvious that the measurement of MRD represents a new and globally working biological principle that can be applied to predict the prognosis of childhood ALL with a given therapy more reliably than any other known method.

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# Reply to the letter from von Stackelberg *et al*

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TO THE EDITOR

We thank von Stackelberg *et al*[1] for their comments. We agree that minimal residual disease (MRD) is emerging as a reliable predictor of outcome in children with first relapse ALL, although some fine tuning to define the optimal time points and cutoff levels to use in each protocol may be necessary. In our series[2], MRD at the end of remission induction was particularly informative in patients who relapsed off-therapy; in our current protocol for first relapse ALL, patients with 'high risk' features are candidates for transplant irrespective of MRD findings. This is in line with the BFM concepts mentioned by von Stackelberg *et al*.

To monitor MRD, we use markers selected from those expressed by the leukemic cells of each patient, akin to the selection of suitable primers for PCR assays. This tailor-made strategy increases the reliability of the assay and ensures that negative findings are not due to the lack of aberrant phenotypes in the leukemic cells. Among the 35 patients monitored for MRD in our study, optimal phenotypes were selected at first relapse in 32, including two with 'isolated' extramedullary relapse: in one of these patients, we analyzed leukemic cells recovered from the spinal fluid; in the other patient, we analyzed those detected by flow cytometry in the bone marrow (which was in morphological remission). Cells were not available at the time of first relapse in only three patients. According to the phenotype determined at diagnosis, one of these patients had MRD at day 36. Of the remaining two patients, both with extramedullary relapse as the first event and MRD-negative at day 36, one had a second extramedullary relapse, with cells retaining the phenotype observed at diagnosis; the other remains in remission. Overall, the immunophenotypes used to monitor MRD were retained in all 14 patients tested at the time of second relapse, including three of the group with undetectable MRD at day 36 (see page 501, second paragraph of Coustan-Smith *et al*[2,8]). To prevent the possibility of phenotypic shift, we always recommend the use of multiple marker combinations whenever possible.[3–6] Likewise, more than one allele-specific oligonucleotide probe should be used with PCR to offset the risk of false-negative results due to oligoclonality and clonal evolution.[7]

Regarding the sensitivity of flow cytometry, one should make a distinction between flow cytometric techniques that rely on combinations that are not expressed by normal hematopoietic cells (including hematogones) and those that use marker combinations that are not completely leukemia specific. We use the former strategy, which is guided by a large number of normal and regenerating reference samples,[5] and yields reliable and clinically useful information.[3,8,9] At our institution, both flow cytometry and PCR have been applied in tandem to select